COMPARATIVE EVALUATION OF SCARDOVIA WIGGSIAE IN CHILDREN BELOW 5 YEARS WITH AND WITHOUT DENTAL CARIES IN RURAL AND URBAN POPULATION OF TERITARY CARE CENTER IN AMMAPETTAI

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1. INTRODUCTION:

1.1.Early childhood caries (ECC):

Early childhood caries (ECC) is a specific form of severe dental caries in infants and young children and it is most prevalent diseases of all childhood diseases. This type of caries initially presents with an early lesion on the smooth surface of primary maxillary incisors and progresses rapidly to other teeth in the oral cavity upon the sequence of tooth eruption. The mandibular incisors are protected from ECC because of the self-cleansing area by the movement of the tongue and salivary flow [1]. Early childhood caries (ECC) is a virulent form of the disease that is a public health problem that continues to affect infants and preschool children worldwide. It is estimated that more than 50% of children with primary teeth suffer from ECC worldwide although epidemiologic studies vary widely across many countries [2]. Historically, severe tooth decay in young children was recognized as nursing caries, nursing bottle syndrome, night bottle mouth, or baby bottle tooth decay [3]. These terms came from the cause of inappropriate bottle-feeding. However, current evidence suggested that bottle nursing was not the only cause of disease, but several factors contributed to the etiology of ECC. The American Academy of Pediatric Dentistry in 2011 defined ECC as the presence of 1 or more decayed, (non-cavitated or cavitated lesions), 1 or more missing teeth (due to caries), or 1 or more filled surfaces (dmfts)in any primary tooth in a child under the age of 6 or younger.

1.2.Severe Early Childhood Caries:

The term "Severe Early Childhood Caries" refers to "atypical", "progressive", "acute", or "rampant" patterns of dental caries. Severe Early Childhood Caries (S-ECC) is defined as caries in children younger than 3 years of age, any sign of smooth-surface caries is indicative of S-ECC [5]. S-ECC considered a significant public health problem both in developing as well as in industrialized countries [6]. The prevalence of S-ECC is reported as 44% in 8- to 48-month-old children [7].

1.3.Risk Factors:

Risk for caries includes factors such as high numbers of cariogenic bacteria, high frequency sugar consumption, inadequate salivary flow, insufficient fluoride exposure, poor oral hygiene, and poverty. The approach to caries prevention should be patient-centered and evidence-based practices regarding the reduction of risk factors and increase in preventive factors.

1.4.Bacterial Agents:

Dental caries is the most common chronic infectious disease of childhood, caused by the interaction of bacteria, mainly Streptococcus mutans, and sugary foods on tooth enamel. S. mutans can spread from mother to baby during infancy and can inoculate even pre-dentate infants. These bacteria break down sugars for energy, causing an acidic environment in the mouth and resulting in demineralization of the enamel of the teeth and dental caries [8]. Streptococcus mutans and Lactobacillus are known as the two most common responsible microorganisms to cause caries [9, 10]. Recent developments in molecular techniques showed that complex bacterial populations like *S.mutans, Veillonella, Actinomyces, Bifidobacterium* and *Lactobacillus* species have been found to be effective in the progress of SECC

1.5.Scardovia wiggsiae:

In 2011, Tanner et al. discovered a new Microbe associated with dental caries, *Scardovia wiggsiae* was named after Vittorio Scardovi, an Italian microbiologist, and Lois Wiggs, an American microbiologist for her contributions to anaerobic microbiology. Scardovia wiggsiae is a gram-positive, anaerobic, non-spore-forming and non-motile bacilli measuring 0.6-0.7μm x 1.6-4μm [11, 12, 13, and 14]. *Scardovia wiggsiae* is acidiogenic /aciduric bacteria, which is predictive of caries and increases the progression of tooth decay [6]. Investigations found highly significant relationship between bacteria like *S. mutans, Scardovia wiggsiae, Veillonella parvula, Streptococcus cristatus, Fusobacterium nucleatum and Actinomyces gerencseriae* with ECC [11]. In addition, significant association of *S. wiggsiae* with ECC has been reported in the presence or absence of other acid-producing microbes, such as Lactobacillus species and *S. mutans* [7]. Although the screening for *Scardovia* are now emerging, the oral microbial ecology and microbial interactions of S. wiggsiae with other oral microorganisms was undiscovered [8].

2. Aim:

To Compare and evaluate the prevalence of *Scardovia wiggsiae* in children below 5 years with and without dental caries in rural and urban population.

3. Objectives:

1. To examine the prevalence of *Scardovia wiggsiae* among pediatric population with and without Early childhood dental caries in Tamilnadu
2. To compare and evaluate the prevalence of *Scardovia wiggsiae* among urban and rural pediatric population.
3. To evaluate the Microbial Ecology of *Scardovia wiggsiae* with other caries associated bacteria.
4. To assess the bacterial diversity in early childhood caries through Metagenomics.

4. Hypothesis/Research Questions:

1. Does the prevalence of *S. wiggsiae* vary between among pediatric population with and without early childhood dental caries?
2. Does *S. wiggsiae* share the same microbial ecology with other caries associated Bacteria?

5. Brief review of literature:

Despite the decline in the prevalence of dental caries in children in western countries, caries in preschool children remains a major problem in both developed and developing countries (9). Prevalence of ECC also varies widely with several factors like race, culture, and ethnicity; socioeconomic status, life style, dietary pattern, and oral hygiene practices and according to the various factors from country to country and from area to area. A review of the literature suggests that in most developed countries the prevalence rate of ECC is between 1 and 12% (10). The prevalence ranged from 11.4% in Sweden to 7–19.0% in Italy (17, 18). A high prevalence of ECC been reported in some Middle Eastern countries, such as Palestine (76%) and the United Arab Emirates (83%) (19, 20). The national surveys from some countries, such as Greece (36%), Brazil (45.8%), India (51.9%), and Israel (64.7%), showed inconsistent prevalence of ECC (11, 12). Dental caries results from the interaction of various etiological factors, which might be concurrently present to initiate and involved in the progress of the disease.

The factors are (1) cariogenic microorganisms, (2) fermentable carbohydrates (substrate), and (3) susceptible tooth surface/host. There are multitudes of risk factors associated with ECC. Epidemiological studies have also documented low socioeconomic status, minority status, low birth weight, and transfer of microbes from mother to child. Child oral health-care behavior,

feeding and cleaning behavior are associated with ECC among children; nighttime bottle-feeding and frequent consumption of cariogenic food; late commencement of child tooth brushing and irregular brushing habits (13). *Streptococcus mutans* (SM) and *Streptococcus sobrinus* are the most common microorganisms associated with ECC. Lactobacilli also participate in the development of caries lesions and play an important role in lesion progression, but not its initiation (14). SM isolates from infants indicated that the source of the SM in children is mainly from their mothers via vertical transmission through saliva (15, 16). Horizontal transmission of microbes may occur between siblings and caregivers. Infants delivered by cesarean section acquire SM earlier than vaginally delivered infants since these deliveries are more aseptic and the atypical microbial environment increases the chances of SM colonization (17).

Streptococcus mutans has been strongly associated with dental caries but caries also occurs in its absence. Association of a new species, *Scardovia wiggsiae* with childhood caries suggests this could be a new caries pathogen. S. wiggsiae was significantly associated with severe-early childhood caries in the presence and absence of S. *mutans*. Further S. wiggsiae was elevated in initial carious lesions in adolescents with fixed orthodontic appliances. S. wiggsiae detection was enriched on a low pH agar suggesting acid-tolerance. S. wiggsiae isolates were acid tolerant and produced acid from several sugars at low initial pH values, and were not arginine deiminase positive, characteristics consistent with potential cariogenicity. Cariogenicity of *S. wiggsiae* tested in a rat animal model in parallel with S. *mutans*. While S. wiggsiae by itself showed minimal caries induction, when co-inoculated with S. *mutans*, there was significant cavity production (18).

Other Scardovia or Scardovia-like species have been associated with dental caries. Thomas and co-workers evaluated caries progression by studying demineralization of enamel chips worn in an intra-oral appliance [19]. S. *mutans* found at higher levels in caries-active than in caries-inactive subjects. Other species detected in the caries-active group included S. *sobrinus*, *Streptococcus intermedius*, several *Lactobacillus* species, *Rothia dentocariosa* and *Scardovia inopinata*, which is genetically close to Scardovia wiggsiae. In another study, *Parascardovia denticolens* cultured from the forefront of carious lesions with vitally exposed pulps suggesting this Scardovia-related species was associated with lesion.

6. Materials and Methods:

6.1.Study population:

100 children below 72 months, who have attended Department of Dentistry and department of Paediatrics, Shri Sathya Sai Medical College and Hospital, Ammapetai, Chengalpattu District,

Tamil Nadu, were enrolled in this study. The study period was determined as 1 year. Inclusion criteria of children were; medically healthy children, having a full primary dentition, and had not used antibiotics within the preceding 4 weeks. Fifty children with ECC 50 children without ECC (Control group) were assessed for the study. Informed consent was obtained from each parent or guardian; and the study procedures were approved Institutional Ethics Committee, Shri Sathya Sai Medical College & Research Institute (2019/519).

6.2.DNA isolation and amplification:

The isolation of DNA from saliva samples was performed using the QIAGEN DNA Purification from Blood or Body Fluids (Spin Protocol) using the manufacturer's recommended protocol. The screening was performed using Bio-Rad Real-time PCR machine (BIORAD CFX96) with the following reaction parameters: Initial Denaturation at 95ºC for 30sec followed by denaturation at 95°C 5 sec and 40 cycles at the annealing (melting) temperatures (Tm) 60°C 30 sec (TB Green™ Premix Ex Taq™ (Tli RNaseH Plus) For the CFX96 Real-Time PCR Detection System).

Positive control used to evaluate the presence of bacteria in sample is 16s rRNA bacterial primer set

Forward 5'-ACGCGTCGACAGACTTTGATCCTGGCT-3'
Reverse 5'- GGGACTACCAGGGTATCTAAT-3'

Species-specific primers used showed in Table 1.

Bacteria	*Universal primers (5'- 3')*	Amplication Length
S. wiggsiae	*GTGGACTTTATGAATAAGC CTACCGTTAAGCAGTAAG*	172bp
S. noxia	TCTGGGCTACACACGTACTACAATG GCCTGCAATCCGAACTGAGA	
S.mutans	*GGTCAGGAAAGTCTGGAGTAAAAGGCTA GCGGTAGCTCCGGCACTAAGCC*	282bp
V. parvula	*GAAGCATTGGAAGCGAAAGTTTCG GTGTAACAAGGGAGTACGGACC*	623bp
F. nucleatum	*AGA GTT TGA TCC TGG CTC AG GTCATCGTGCACACA GAA TTG CTG*	360bp

Table 1: Universal primers used in PCR analysis

6.3.Metagenomics analysis:

6.3.1. Library preparation:

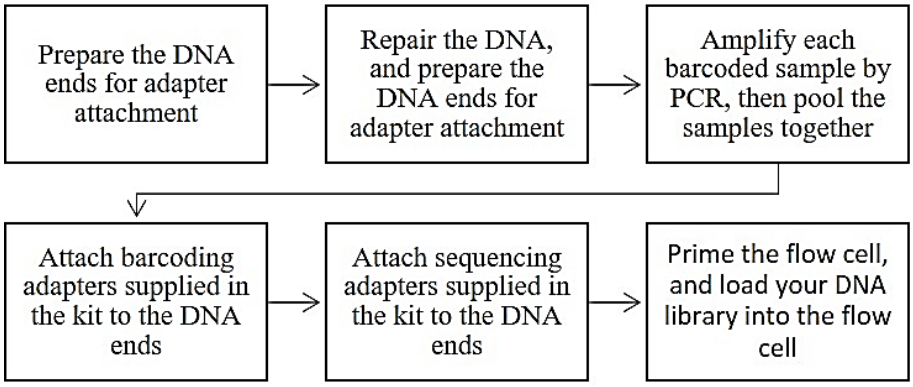
Prepare the DNA ends for adapter attachment
Repair the DNA, and prepare the DNA ends for adapter attachment
Amplify each barcoded sample by PCR, then pool the samples together
Attach barcoding adapters supplied in the kit to the DNA ends
Attach sequencing adapters supplied in the kit to the DNA ends
Prime the flow cell, and load your DNA library into the flow cell

Figure 1. Showing the Library preparation for metagenomics analysis

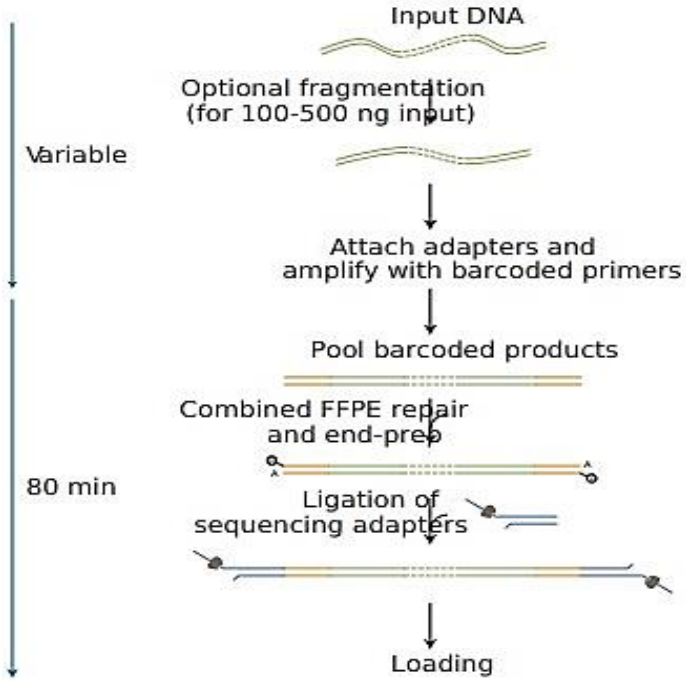

Figure 2: Showing the library preparation process

6.3.2. Software for Nano pore sequencing

1. **MinKNOW:** The MinKNOW software controls the nanopore-sequencing device, collects sequencing data in real time and processes it into base calls.
2. **EPI2ME** (optional): The EPI2ME cloud-based platform performs further analysis of base called data.
3. **Guppy** (optional): The Guppy command-line software used for base calling and de-multiplexing reads by barcode instead of MinKNOW. You can use it if you would like to re-analyse old data, or integrate base calling into your analysis pipeline.

7. Results & Discussion:

The prevalence of S. wiggsiae, S. noxia, S. mutens, and V.parvula was found to be significantly higher in the ECC group than in the control group. There was no statistically significant difference between F. nucleatum and S.mutens within the two groups (P>0.05). S. wiggsiae and S.noxia, S. wiggsiae and S. mutans, S. wiggsiae and F. nucleatum were found to be significantly more prevalent in the ECC group than in the control group (P: 0.001; P0.01). S. wiggsiae, S.noxia, S. mutans, F. nucleatum, and V parvula account for 10% of the total.

Because there is little information about the prevalence of the novel cariogenic bacterium S.wiggsiae and its relationship with other oral microbiota, the major goal of this study was to screen for S.wiggsiae and assess the microbial flora present alongside S.wiggsiae. Modern molecular genetic analysis has been developed to improve perception of oral microbiota since it takes less time than the classic microbiological identification methodology [20]. The development of molecular tools such as PCR has resulted in a new revolution in finding distinct and novel cariogenic bacteria. Vacharaksa et al. (2015) underlined that novel molecular detection approaches can identify complex bacteria communities that play a major role in dental caries [21].

Munson et al. [22] discovered 44 taxa of cariogenic bacteria in dentinal carious lesions, 31 of which had previously gone unnoticed. Real-time PCR using species-specific 16SrRNA primers detects individual bacterial populations accurately and sensitively [24]. Real-time PCR analysis using species-specific 16SrRNA primers employed in this investigation to detect selected oral bacteria.

S. wiggsiae, a new cariogenic bacteria belonging to the *Bifidobacteriaceae* family, was

recently identified as a key component of the early childhood dental caries-related microbial complex [30]. Furthermore, *S. wiggsiae* is frequently identified in white spot lesions; it is thought that *S. wiggsiae* contributes in the development of enamel lesions, hence producing caries [25]. By demonstrating a higher prevalence and abundance of *S. wiggsiae* in children with ECC compared to caries-free children, Vacharaksa et al. emphasize the importance of S. wiggsiae in caries development and progression [21]. This backs up our findings that S. wiggsiae was more prevalent in children with ECC than in children without ECC.

Even though *S. mutans* is considered to be the major pathogen of dental caries, it was also found in people who do not have caries. *S. wiggsiae* causes ECC in paediatrics in both the presence and absence of *S. mutans*, according to research [30]. *S. mutans* was found more frequently than S. wiggsiae in our study, while S. wiggsiae was found even when *S. mutans* was not present. This shows that these novel cariogenic bacteria were not related to ECC.

Another belief is that *S. mutans* is not the dominant species in the caries microbiota as previously mentioned, and that *S. wiggsiae* is a secondary inhabitant associated with the spread of caries into the dentin in later stages of infection [30]. In 2020, Kameda et al. discovered that *S. wiggsiae* had high acidogenic and aciduric characteristics, making it ecologically competitive in acidic settings such as caries lesions, where lactic acid predominates. [26]

Tanner et al. (2012) reported that, in addition to *S. mutans* and *S. wiggsiae*, *V. parvula*, *S. cristatus*, *F. nucleatum*, and *A. gerencseriae* are linked to ECC [24]. Our study found that children with ECC had a considerably higher prevalence of S. wiggsiae, S. noxia, S. mutans, and F. nucleatum, whereas children without ECC have a higher prevalence of F. nucleatum and S. mutans. In contrast to the previous study, there is no significant difference in F. nucleatum and S. mutans in children with and without ECC. Although the prevalence of V. parvula is low in children with ECC, it is higher in children with ECC than in children without ECC.

S-ECC is linked to combinations of *S. wiggsiae* and *S. mutans*, *S. sobrinus* and *S. mutans*, and *Bifidobacteriaceae* and S. mutans, according to species-specific PCR in children [30]. In children with ECC, the combination of S. wiggsiae and S. noxia, S. wiggsiae and S. mutans, and *S. wiggsiae* and *F. nucleatum* is much greater. *F. nucleatum*, on the other hand, is significantly greater in both ECC and caries-free children, confirmed by a study that revealed Fusobacterium species at high levels in persons with healthy oral cavity and early-phase caries lesions [27]. The presence of F. nucleatum in children with ECC (100%) and children without ECC (98%) shows that F. nucleatum is a persistent part of the natural microbiota.

This study found a greater and equal association rate between *S. wiggsiae* and *S. noxia*, as well as *S. wiggsiae* and *F. nucleatum* (40%) in children with ECC. S.noxia is a common microbial flora found in the gastrointestinal tract and the mouth cavity. *S.noxia* levels are higher in patients with poor oral health [28, 29]. Researchers believe that S. wiggsiae and S. noxia may occupy unique, non-overlapping niches [31], in contrast to our study, which found that S. wiggsiae and S. noxia share 40% of the same microbial ecology. The combination of *S.wiggsiae* with *S. noxia*, *S.mutans*, *F.nucleatum*, and *V.Parvula* is minor (7%), which may be due to each child's different oral ecological balance.

Metagenomic analysis is a DNA sequencing technique, which gives us an insight about taxonomic diversity. Descriptive level of taxonomic resolution or previously determined bacteria of similar description are sequenced using microarray method. 16S rRNA gene sequencing used in multiple taxonomic stints, as in HOMD, the common database for oral bacteria, been established as the "gold standard" for identification of bacterial species. It serves as a rapid and reliable alternative method for bacterial identification quantitatively.

Furthermore, in order to determine the bacterial population in the dentinal carious, genomic analysis of dentinal sample was analysed. Data analysis showed presence of varying percentage of Lactobacilliles for Order, whereas streptococcus sp. was observed in abundance than *flavobacteriacea* and *enterococococea* among taxa families. Presence of S. wiggsiae was observed in low number of sample tested being obligate anaerobe from the saliva and it needs species-specific primers to like used for PCR. Almost all the samples carried Lactobacillus (11%–95%). The screening of Lactobacilli in this study is consistent with the previous reports [32, 33]. In metagenomics analysis of 16S rRNA gene, the taxonomic resolution below descriptive level cannot be analysed, hence the species determination of the bacteria was not possible in this study.

This study's population of children was limited to 100 in Tamilnadu, India. There is a need for study in diverse places and with a wider range of participants to better understand the acquisition and transmission of S. wiggsiae and other oral bacteria in ECC. However, there have been little investigations on S. wiggsiae, and its clinical consequences remain unknown.

8. Conclusion:

These results demonstrated several novel findings, as *S. wiggsiae* is associated with the ecology of ECC. The study has shown that, not only a single cariogenic bacterium is responsible for ECC, but also a gamut of oral microbiota may support for the development and progression of caries. S.wiggsiae is significantly higher in children with ECC and is one among the predominant

bacteria in Children from underprivileged and low socioeconomic status. A wide range of risk factors is associated with ECC in children from underprivileged and low socioeconomic status. Oral health has been recognized as an essential component of general health and quality of life. Hence, both oral disease prevention and oral health promotion should be included as an integral part of chronic disease prevention and general health promotion programs. Further studies may be required to understand the role of microbiota in caries etiology, evolution and their clinical implications.

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